SEPARATION OF TWO DIFFERENT PEPTIDYL TRANSFER RNA TRANSLOCASES FROM MAMMALIAN TISSUES

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1. Introduction

Two different enzymes are required for the elongation of peptide chains on mammalian ribosomes. The binding enzyme [1] or transferase I [2] binds aminoacyl-tRNA to the acceptor site of ribosome. Transferase II [3] translocates the peptidyl-tRNA from the donor to the acceptor site. Multiple forms of the binding enzyme seem to exist in rat liver [4]. However, only a single translocase was so far reported to be present in mammalian cells.

Several batches of elongation factors were recently isolated in this laboratory using the method of Bermek and Matthaei [5, 6]. During the purification of transferase II on DEAE cellulose columns, the bulk of translocase activity was eluted with 100 mM KCl. However, a low but constant transferase II activity appeared in the eluate when the concentration of KCl was increased to 200 mM. These results indicate that two different translocases exist in mammalian tissues.

This paper described the isolation of two translocases from rat liver and human tonsils differing in their molecular weight. Data are also presented indicating that both these enzymes have complementary activities.

2. Materials and methods

Both peptide elongation factors were precipitated from rat liver or human tonsil cell sap by $(NH_4)_2SO_4$ and transferase I was separated from transferase II by passage through Sephadex G-200 columns as described by Bermek and Matthaei [5, 6]. The binding enzyme was further purified by Sepharose 4B chromatography according to the same authors. Both translocases were separated on DEAE-cellulose columns as indicated. Ribosomes from both tissues were purified by centrifugation in discontinuous sucrose gradients followed by washings with NH_4Cl [7]. Incubation mixtures for the assay of polyU-dependent polyphenylalanine synthesis contained tris-HCl, pH 7.40, 50 mM; $MgCl_2$, 11 mM; KCl, 55 mM; 2-mercaptoethanol, 10 mM; GTP, 2 mM; polyU, 20 μg; homologous ribosomes, 80 μg; rat liver ^{14}C-Phe-tRNA (5.9 μmoles Phe/mmole of tRNA), 25 μg (or equivalent quantities of human tonsil ^{14}C-Phe-tRNA, 8.5 μmoles Phe/mmole of tRNA)); saturating amounts of the homologous binding enzyme (3–5 μg of protein) and quantities of both translocases as indicated, in a total volume of 0.1 ml. Mixtures were incubated and proteins prepared for the assay of radioactivity as described earlier [8]. Ultracentrifugal analyses were carried out using the MOM 3170 ultracentrifuge (MOM, Budapest, Hungary) at 50,000 rpm and 20°.

3. Results

3.1. *Chromatographic separation of both translocases*

When proteins precipitated with $(NH_4)_2SO_4$ from rat liver or human tonsil cel sap were subjected to gel filtration on Sephadex G-200 columns, a good separation of transferase I from transferase II was obtained in all instances. Moreover, two peaks of translocase activity were found with some preparations at this stage (fig. 1).

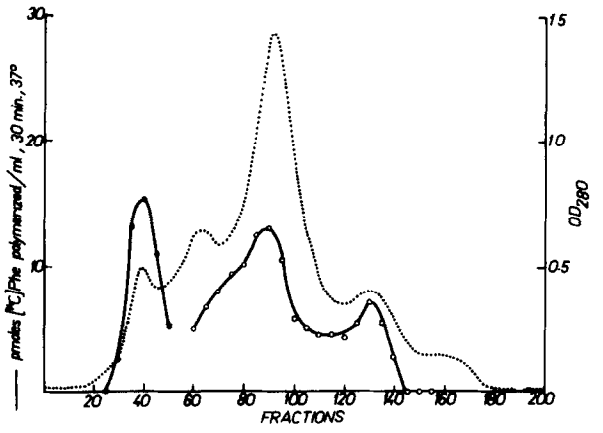

Fig. 1. Separation of peptide elongation factors from human tonsils on Sephadex G-200. Proteins precipitated with $(NH_4)_2SO_4$ from the postmicrosomal supernatant of 31 g of human tonsils [5, 6] were applied on a Sephadex G-200 column (40 X 750 mm) equilibrated with the standard buffer containing 100 mM of KCl [8] and eluted with the same buffer. Fractions of 4.5 ml were collected. Aliquots of fractions no. 20–50 (20 μl) were tested for transferase I activity in the standard polymerization assay with saturating quantities of purified homologous transferase II fraction IV [8]; 25 μl aliquots of fractions 50–150 were tested in the same way with saturating amounts of transferase I fraction IV [5, 6].

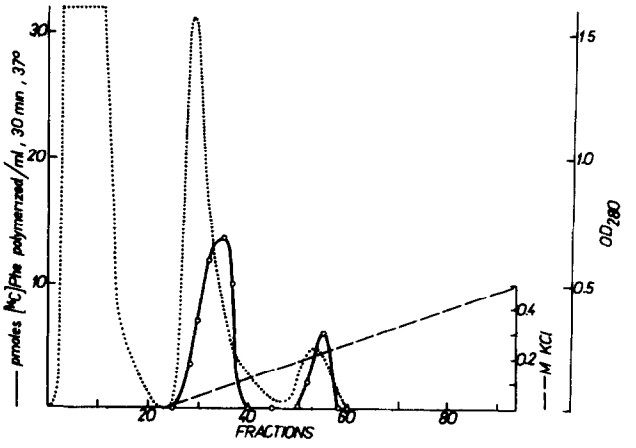

Fig. 2. Separation of both peptidyl-tRNA translocase activities from rat liver on DEAE-cellulose. The crude translocase fraction after chromatography on Sephadex G-200 dialyzed against the standard buffer [8] was applied on a column of DEAE-cellulose (15 X 190 mm) equilibrated with the same buffer. The non-adsorbed protein was eluted with the standard buffer and after that a linear gradient 0–500 mM of KCl was applied. 9.5 ml fractions were collected. The translocase activity of aliquots (25 μl) of all fractions was tested in the standard polymerization assay with saturating amounts of homologous transferase I fraction IV [5, 6].

Since only incomplete separations of both transferases II could be obtained in this way, all fractions with translocase activity were pooled, dialyzed against standard buffer [8], concentrated with Aquacide II (Calbiochem, Luzerne, Switzerland) and applied on DEAE-cellulose (Whatman DE-50) columns equilibrated with standard buffer. The bulk of proteins present in this fraction was not retained on the column. A complete separation of both translocases was obtained when a linear gradient 0–0.5 M KCl was applied (fig. 2).

The yields of transferase IIb were only 0.1–0.15 mg/g of tissue. Essentially the same results were obtained with elongation factors isolated from rat liver (3 preparations) as with those from human tonsils (2 batches).

In the ultracentrifuge the translocase fraction eluted at about 80 mM KCl (TF IIa) showed a main peak accounting for at least for 80% of the total protein with $S_{20,w}$ = 4.4 S. In addition, traces of three proteins with a higher molecular weight were present in these preparations. Proteins eluted at about 200 mM KCl (transferase IIb) showed only a single peak in the ultracentrifuge with $S_{20,w}$ of 2.6 S. Since the concentration of transferase IIb used for the analyses was rather low, the presence of additional impurities cannot be excluded.

3.2. *Activity of both translocases in the polymerization assay*

Both translocases showed an absolute requirement for the binding enzyme and no polymerization of phenylalanine occurred in the absence of transferase I.

The activity of separated transferases II was considerably lower than that of unseparated TF II preparations eluted from DEAE-cellulose with 0.2 M KCl used in our previous experiments [8]. The same phenylalanine polymerization was obtained in homologous systems when saturating amounts of TF IIa or TF IIb were used. However, the saturating amount of TF IIa lies in the range of 0.25–0.30 mg of protein/ml of incubation mixture, whereas only about 0.06 mg

Table 1
Effect of rat liver transferase IIa and IIb on the polyphenylanaline synthesis in rat liver systems.

Transferase II protein (μg/ml)		^{14}C-Phe polymerized (pmoles/ml, 30 min, 37°)
TF IIa	TF IIb	
65	–	6.0
–	4	0.6
–	10	1.6
65	4	7.5
65	10	9.5

Incubation mixtures contained saturating amounts of rat liver binding enzyme (32 μg of TF I fraction IV proteins/ml) [8] and all other components described for the standard polymerization assay.

of protein/ml are required for the saturation when TF IIb is used. The saturation curves for both enzymes indicate that TF IIb has a significantly higher specific activity than the other translocase.

Polyphenylalanine synthesis in reaction mixtures containing both translocases was not a mere summation of activities of both enzymes. Addition of minute quantities of transferase IIb significantly stimulated the activity of transferase IIa (table 1). Similarly the addition of TF IIa enhanced the polyphenylalanine synthesis catalyzed by TF IIb. Essentially the same results were obtained with enzymes from rat liver as with those from human tonsils.

The TF IIa: TF IIb ratio in incubation mixtures was apparently of critical importance for maximal phenylalanine polymerization. Thus additions of an excess of one transferase lead to a significant inhibition of polyphenylalanine synthesis (fig. 3).

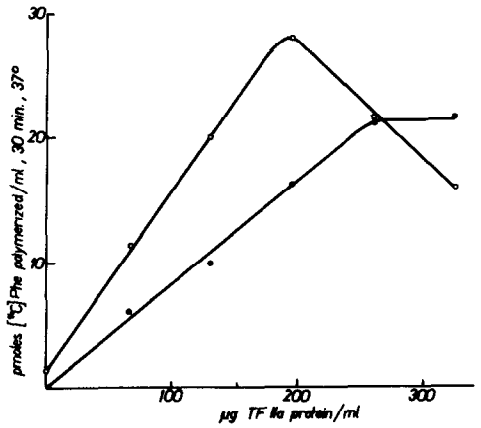

Fig. 3. Effect of increasing amounts of transferase IIa alone (●——●) or in combination with transferase IIb (8 μg of protein/ml) (○——○) on the polyU-directed polyphenylalanine synthesis in the rat liver system.

4. Discussion

A complex of both peptide elongation factors that can be separated from individual transferases does apparently exist in yeast [9]. However, neither of translocases described in this paper can represent such a complex since both these enzymes show an absolute requirement for transferase I. Two sets of transfer factors, including two G factors corresponding to transferase II, were isolated from algae [10]. However, the activity of these factors is strictly limited to individual types of ribosomes and they do not show any complementary effect.

In the method of Bermek and Matthaei [5, 6] the postmicrosomal supernatant is used as the starting material for the isolation of both elongation factors whereas most authors isolate mammalian transfer factors from pH 5 supernatants. This may be why the existence of a translocase corresponding to transferase IIb has not yet been reported. This enzyme may easily be coprecipitated with pH 5 enzymes or may be destroyed during the acidification of the cell sap.

The sedimentation behavior of transferase IIa corresponds to that of the translocase purified by Moldave's group from rat liver for which an $S_{20,w} = 4.7$ S has been reported [11]. It seems probable that both these enzymes are identical. On the other hand, the significance of TF IIb is not clear at the moment. Its lower molecular weight may indicate that this enzyme represents active subunits of the other translocase. The binding enzyme of rat liver is apparently dissociated into such active subunits [4] and it may be the case with translocase also. Alternatively, TF IIb may be a different enzyme from TF IIa

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